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<110> Skeiky, Yasika Guderian, Jeffferank Corixa Corporation

<120> Methods of Using a Mycobacterium tuberculosis Coding Sequence to Facilitate Stable and High Yield Expression of Heterologous Proteins

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<140> US 09/684,215

<141> 2000-10-06

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<151> 1999-10-07

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TECH CENTER 1600/2900

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Ay Auri

25

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            20
                                25
Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
        35
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Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
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Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala Glu Phe Ile Glu Gly
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                                           140
Arg Gly Ser Gly Cys Pro Leu Leu Glu Asn Val Ile Ser Lys Thr Ile
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145
                  150
Asn Pro Gln Val Ser Lys Thr Glu Tyr Lys Glu Leu Leu Gln Glu Phe
                                    170
Ile Asp Asp Asn Ala Thr Thr Asn Ala Ile Asp Glu Leu Lys Glu Cys
           180
                               185
Phe Leu Asn Gln Thr Asp Glu Thr Leu Ser Asn Val Glu Val Phe Met
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Gln Leu Ile Tyr Asp Ser Ser Leu Cys Asp Leu Phe
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<210> 11
<211> 2191
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:Ra12-H9-32A fusion
      (Ra12-MTB39-MTB32A(N-ter) fusion)
<220>
<221> CDS
<222> (1) .. (2190)
<223> Ra12-H9-32A (Ra12-MTB39-MTB32A(N-ter)) fusion polypeptide
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Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
tcc cag ggt ggg cag gga ttc gcc att ccg atc ggg cag gcg atg gcg
Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
             20
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atc Ile	gcg Ala	ggc Gly 35	cag Gln	atc Ile	cga Arg	tcg Ser	ggt Gly 40	ggg Gly	999 Gly	tca Ser	ccc Pro	acc Thr 45	gtt Val	cat His	atc Ile	144
ggg Gly	cct Pro 50	acc Thr	gcc Ala	ttc Phe	ctc Leu	ggc Gly 55	ttg Leu	ggt Gly	gtt Val	gtc Val	gac Asp 60	aac Asn	aac Asn	ggc Gly	aac Asn	192
ggc Gly 65	gca Ala	cga Arg	gtc Val	caa Gln	cgc Arg 70	gtg Val	gtc Val	ggg Gly	agc Ser	gct Ala 75	ccg Pro	gcg Ala	gca Ala	agt Ser	ctc Leu 80	240
ggc Gly	atc Ile	tcc Ser	acc Thr	ggc Gly 85	gac Asp	gtg Val	atc Ile	acc Thr	gcg Ala 90	gtc Val	gac Asp	ggc Gly	gct Ala	ccg Pro 95	atc Ile	288
aac Asn	tcg Ser	gcc Ala	acc Thr 100	gcg Ala	atg Met	gcg Ala	gac Asp	gcg Ala 105	ctt Leu	aac Asn	ggg Gly	cat His	cat His 110	ccc Pro	ggt Gly	336
gac Asp	gtc Val	atc Ile 115	tcg Ser	gtg Val	acc Thr	tgg Trp	caa Gln 120	acc Thr	aag Lys	tcg Ser	ggc Gly	ggc Gly 125	acg Thr	cgt Arg	aca Thr	384
999 Gly	aac Asn 130	gtg Val	aca Thr	ttg Leu	gcc Ala	gag Glu 135	gga Gly	ccc Pro	ccg Pro	gcc Ala	gaa Glu 140	ttc Phe	atg Met	gtg Val	gat Asp	432
ttc Phe 145	ggg Gly	gcg Ala	tta Leu	cca Pro	ccg Pro 150	gag Glu	atc Ile	aac Asn	tcc Ser	gcg Ala 155	agg Arg	atg Met	tac Tyr	gcc Ala	ggc Gly 160	480
	ggt Gly															528
gcg Ala	agt Ser	gac Asp	ctg Leu 180	ttt Phe	tcg Ser	gcc Ala	gcg Ala	tcg Ser 185	gcg Ala	ttt Phe	cag Gln	tcg Ser	gtg Val 190	gtc Val	tgg Trp	576
ggt Gly	ctg Leu	acg Thr 195	gtg Val	ggg Gly	tcg Ser	tgg Trp	ata Ile 200	ggt Gly	tcg Ser	tcg Ser	gcg Ala	ggt Gly 205	ctg Leu	atg Met	gtg Val	624
	gcg Ala 210															672
	gag Glu															720
	gcg Ala															768
	gaa Glu															816

					•											
					gtt Val											1584
					atc Ile											1632
gcc Ala 545	gac Asp	ttc Phe	ccc Pro	gcg Ala	ctg Leu 550	ccc Pro	cțc Leu	gac Asp	ccg Pro	tcc Ser 555	gcg Ala	atg Met	gtc Val	gcc Ala	caa Gln 560	1680
					gtc Val											1728
					acc Thr											1776
					gtg Val	Ile										1824
					caa Gln											1872
_		_	•	_	gcg Ala 630		_	_	_	-					_	1920
					ggt Gly											1968
					ggt Gly											2016
					ctc Leu											2064
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~ ~	-		_		acg Thr	_			tag 730	g	•					2191

ally.

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<210> 12
<211> 729
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:Ra12-H9-32A fusion
     polypeptide (Ral2-MTB39-MTB32A(N-ter) fusion polypeptide)
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Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
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 1
                 5
Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
            20
                                25
Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
                       55
                                           60
Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
                                       75
                    70
Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
                                   90
                85
Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
                              105
                                                  110
          100
Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr
                                              125
                           120
       115
Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala Glu Phe Met Val Asp
   130
                     135
                                          140
Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly
                  150
                                      155
Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val
               165
                                  170
                                                       175
Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp
           180
                              185
                                                  190
Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val
                           200
                                              205
       195
Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln
                      215
                                          220
Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu
                  230
                                       235
Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg
              245
                                  250
Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr
           260
                              265
Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln
                           280
       275
Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr
                      295
                                          300
Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly
                   310
                                      315
Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala
               325
                                   330
                                                       335
Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu
          340
                             345
                                                 350
Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu
                          360
       355
Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn Met Val Ser
                      375
                                          380
Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr
385
                  390
                                     395
Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala
               405
                                   410
                                                      415
```

Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser 420 425 Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Val Ala Ala 440 Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala 455 460 Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro 470 465. 475 Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu 490 Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu 510 500 505 Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser 520 Pro Ala Ala Gly Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe 535 540 Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln 550 Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn 565 570 Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val 585 590 Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe 605 600 Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp 615 Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu 635 Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val 645 650 Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro 665 Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu 680 Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala 695 700 Ile Gln Pro Gly Asp Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln 710 715 Val Val Gly Met Asn Thr Ala Ala Ser 725

<210> 13

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer for PCR amplification of Ra12 C-terminal fragment of MTB32A

<400> 13

caattacata tgcatcacca tcaccatcac acggccgcgt ccgataactt c

51

<210> 14

<211> 33

<212> DNA

<213> Artificial Sequence

<220> <223> Description of Artificial Sequence:3' oligonucleotide primer for PCR amplification of Rall C-terminal fragment of MTB32A 33 ctaatcgaat tcggccgggg gtccctcggc caa <210> 15 <211> 48 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence:5' oligonucleotide primer containing enterokinase recognition site for PCR amplification of DPPD mature secreted form caattagaat togacgacga cgacaaggat ccacctgacc cgcatcag 48 <210> 16 <211> 33 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence:3' oligonucleotide primer containing enterokinase recognition site for PCR amplification of DPPD mature secreted form 33 caattagaat totcagggag cgttgggctg ctc <210> 17 <211> 30 <212> PRT <213> Artificial Sequence <220> <223> Description of Artificial Sequence:Ra12(short) polypeptide <400> 17 Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe 10 Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile <210> 18 <211> 128 <212> PRT <213> Artificial Sequence

<220> <223> Description of Artificial Sequence:Ral2(long) polypeptide <400> 18 Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe 1 Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala 60 Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val 75 65 70 Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn 90 Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser 105 110 Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala 125 <210> 19 <211> 34 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence:5' oligonucleotide primer, HindIII site, for PCR amplification of human mammaglobin <400> 19 34 gcgaagctta tgaagttgct gatggtcctc atgc <210> 20 <211> 36 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence:3' oligonucleotide primer, XhoI site, for PCR amplification of human mammaglobin <400> 20 cggctcgagt taaaataaat cacaaagact gctgtc 36 <210> 21 <211> 7 <212> PRT <213> Artificial Sequence

<223> Description of Artificial Sequence: Met-His tag 6aa

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<400> 21
Met His His His His His His
1 5
<210> 22
<211> 4
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<212> PRT <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:enterokinase
 recognition site

<400> 22 Asp Asp Asp Lys

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Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr
                                         75
Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val
                                     90
Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln
                                105
           100
Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala
                           120
      115
Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly
                                            140
                        135
Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
                                        155
                   150
Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
                165
                                    170
Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
                                185
Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
        195
                            200
Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
                                            220
                        215
Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe Ala
                                        235
                   230
Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser Gly
                                    250
                245
Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu
                                265
Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val
                            280
Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile
                                            300
                        295
Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp
                                        315
                    310
Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln
                                    330
Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly
                                345
Pro Pro Ala
        355
<210> 3
<211> 396
<212> DNA
<213> Mycobacterium tuberculosis
<223> 14 KD C-terminal fragment of MTB32A Ral2
<220>
<221> CDS
<222> (1)..(396)
<223> Ra12
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Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe
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gcc Ala	att Ile	ccg Pro	atc Ile 20	Gly ggg	cag Gln	gcg Ala	atg Met	gcg Ala 25	atc Ile	gcg Ala	ggc Gly	cag Gln	atc Ile 30	cga Arg	tcg Ser	96
ggt Gly	Gly Gly	999 Gly 35	tca Ser	ccc Pro	acc Thr	gtt Val	cat His 40	atc Ile	ggg Gly	cct Pro	acc Thr	gcc Ala 45	ttc Phe	ctc Leu	ggc Gly	144
ttg Leu	ggt Gly 50	gtt Val	gtc Val	gac Asp	aac Asn	aac Asn 55	ggc Gly	aac Asn	ggc Gly	gca Ala	cga Arg 60	gtc Val	caa Gln	cgc Arg	gtg Val	192
gtc Val 65	GJA aaa	agc Ser	gct Ala	ccg Pro	gcg Ala 70	gca Ala	agt Ser	ctc Leu	ggc Gly	atc Ile 75	tcc Ser	acc Thr	ggc Gly	gac Asp	gtg Val 80	240
atc Ile	acc Thr	gcg Ala	gtc Val	gac Asp 85	ggc Gly	gct Ala	ccg Pro	atc Ile	aac Asn 90	tcg Ser	gcc Ala	acc Thr	gcg Ala	atg Met 95	gcg Ala	288
gac Asp	gcg Ala	ctt Leu	aac Asn 100	Gly ggg	cat His	cat His	ccc Pro	ggt Gly 105	gac Asp	gtc Val	atc Ile	tcg Ser	gtg Val 110	acc Thr	tgg Trp	336
caa Gln	acc Thr	aag Lys 115	tcg Ser	ggc Gly	ggc Gly	acg Thr	cgt Arg 120	aca Thr	ggg Gly	aac Asn	gtg Val	aca Thr 125	ttg Leu	gcc Ala	gag Glu	384
	ccc Pro 130	_	_													396
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<22 <22	0> 3> 1	4 KD	C-t	ermi	nal	fraġ	ment	of	мтвз	2A R	a12			·		
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1				5					10				Ile	15 Arg		
			20				His	25	v			Ala	30	i	Gly	
Leu				Asp	Asn			Asn	Gly	Ala			Gln	Arg	Val	
	50 Gly		Ala	Pro				Leu	Gly	Ile			Gly	Asp	Val 80	
65 Ile		Ala	Val	Asp 85			Pro	Ile	Asn 90	Ser		Thr	Ala	Met 95	Ala	
Asp	Ala	Leu	Asn 100	Gly		His	Pro	Gly 105	Asp		Ile	Ser	Val	Thr	Trp	
Gln	Thr	Lys 115	Ser		Gly	Thr	Arg	Thr		Asn	Val	Thr 125	Leu		Glu	
Glv	D-0	Pro														

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<400> 17
Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe
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Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile
<210> 18
<211> 128
<212> PRT
                                                     full longer
<213> Artificial Sequence
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      polypeptide
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                                     10
Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Lys Leu
                                 25
             20
Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val
                             40
         35
Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala
                         55
                                             60
Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val
                                         75
Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn
                                     90
Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser
                                105
Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala
                            120
        115
<210> 19
<211> 34
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:5'
      oligonucleotide primer, HindIII site, for PCR
      amplification of human mammaglobin
<400> 19
                                                                    34
gcgaagctta tgaagttgct gatggtcctc atgc
<210> 20
<211> 36
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:3'
      oligonucleotide primer, XhoI site, for PCR
      amplification of human mammaglobin
<400> 20
                                                                    36
cggctcgagt taaaataaat cacaaagact gctgtc
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<210> 21
<211> 7
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Met-His tag 6aa
<400> 21
Met His His His His His
<210> 22
<211> 4
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:enterokinase
      recognition site
<400> 22
Asp Asp Asp Lys
<210> 23
<211> 128
<212> PRT
<213> Mycobacterium tuberculosis
<223> positions 1-128 of Ra12
<400> 23
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                  5
 1
Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser
                                 25
            20
Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly
                             40
Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val
                         55
Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val
                     70
Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala
                                     90
                 85
Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp
                                                    110
                                105
Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu & NO 4 + 64 Pr Pro Ala
                            120
```